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Reproductive plasticity and local adaptation in the host–parasite system formed by the toxic *Alexandrium minutum* and the dinoflagellate parasite *Parvilucifera sinerae*

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ABSTRACT

A parasite threat stimulates adaptive shifts in the life-history strategy (sexual recombination rate) of the toxic bloom-forming dinoflagellate *Alexandrium minutum* Halim. This microalgae divides asexually when clonal but can also form mobile zygotes (planozygotes) when compatible clones are crossed. Planozygotes usually form resistant dormant stages (resting cysts) although they can also divide. In this study, asexual and sexual cultures were infected with the parasite *Parvilucifera sinerae* (Perkinsozoa) and the resulting clones classified as susceptible (S), low susceptible (LS), or resistant (R) to the infection. R and LS clones were never of Mediterranean origin, pointing to local adaptation of the parasite. (S × S) crosses were infected faster than either of the parental clones growing asexually. By contrast, (S × R) crosses were resistant to the parasite and produced no resting cysts, even when planozygotes were formed. Therefore, in infected cultures, the planozygotes mainly divided instead of encysting, thus increasing the rate at which recombinant progeny formed. This strategy against infection seems to combine the benefits of quickly producing asexual offspring and increasing recombination. As the susceptibility of the crosses was dependent on parental sexual compatibility, and cultures established by the division of (R × S) planozygotes (F1 offspring) also formed R or LS cultures, resistance may be regulated by several genes or through maternal effects.

1. Introduction

Reproductive plasticity equips organisms with the ability to produce phenotypes that are thought to confer a high degree of fitness in response to changes in environmental conditions during their lifetimes (Stearns, 1992). This kind of plasticity has been described in mammals (e.g., Reilly et al., 2006), invertebrates (e.g., McGovern, 2003), fish (e.g., Aubin-Horth and Dondson, 2004), and plants (Barot et al., 2005) but, to our knowledge, never in organisms capable of both sexual and asexual reproduction. This dual life-cycle pattern makes these organisms very interesting from an evolutionary point of view as during parasite attack it necessitates a choice between faster reproduction and the emergence of genetically different offspring.

Dinoflagellates are haploid, unicellular algae that divide asexually but also undergo sexual reproduction. Under laboratory conditions, sexual differentiation occurs in response to nitrogen and/or phosphorous stress and involves the formation of a mobile zygote (planozygote), which usually develops into a resistant dormant stage (resting cyst). After a mandatory period of dormancy, the resting cyst initiates meiosis, giving rise to four recombinant haploid products that resume vegetative growth. Alternatively, under certain environmental conditions, a young planozygote undergoes meiosis, divides, and resumes vegetative growth as above but without forming a resting cyst (Figueroa and Bravo, 2005a,b; Figueroa et al., 2006a,b, 2007). This life cycle provides a relatively simple method to determine not only whether sexuality is triggered by a particular stimulus (which can be checked by measuring the percentage of planozygotes formed), but also whether sexual recombination rate will be faster or a slower (planozygote division giving rise to recombinant progeny is faster than planozygote encystment because the dormancy period of resting cysts can be of several months duration). Dinoflagellates can also form asexual cysts (hereafter referred to as pellicle cyst based on Bravo et al., 2010), which have no dormancy period and are much less resistant against external threats. Although Toth et al. (2004) reported life-cycle changes in a dinoflagellate species under parasite attack, their study was limited to the changes occurring in the asexual cycle. Specifically, the formation of asexual benthic stages, also called temporary cysts, was observed to increase in the presence of the parasite *Parvilucifera infectans*.

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However, it is unknown whether the complex sexual cycle of these protists also has a plastic response to parasite attack, and if so, in which direction the asexual–sexual ratio is modified.

Photosynthetic dinoflagellates are important primary producers in marine ecosystems but some bloom-forming species are able to produce toxins that are potentially lethal in humans (Zingone and Enevoldsen, 2000). Recent observations suggest that although bloom-forming dinoflagellates escape control by grazing organisms, they may eventually succumb to parasite attack, given that a specific host species is infected by a single, genetically distinct parasite species year after year (Chambouvet et al., 2008). Under such conditions, host and parasite gene frequencies in a population are critical in determining the selective outcome, with frequency-dependent selection and local adaptation playing important auxiliary roles in this environmentally relevant system (Carius et al., 2001).

Nevertheless, the occurrence of local adaptation is dependent on the amount of gene flow in host and in parasite populations as well as on parasite virulence and the degree of genetic specificity for the infection (Gandon et al., 1996; Lively, 1999; Gandon, 2002). A combination of these factors may explain why although parasites have been reported to be more infective to sympatric hosts (locally adapted) in many cases (for e.g., Parker, 1985; Lively, 1989; McCoy et al., 2002; Osnas and Lively, 2004), no difference in infectivity on sympatric versus allopatric hosts, or that parasites are less infective to sympatric hosts, has also been reported (for e.g., Roy, 1998; Mutikainen et al., 2000; Kraaijeveld and Godfray, 2001).

Additionally, selection, population structure, and the impact of parasitism on host populations can be modulated by phenotypically plastic life-history traits, such that, for example, the fitness consequences of infection are reduced. Therefore, to determine the evolutionary significance of parasitism it is important to establish the relative contributions of both genetic and phenotypically plastic factors to the expression of an infection (Chadwick and Little, 2005). In the present study, we analyzed host life-history shifts in a system comprising the dinoflagellate species *Alexandrium minutum* Halim and the perkinsozoid parasite *Parvilucifera sinerae* Figueroa and Garcés.

Blooms of the dinoflagellate species *A. minutum* have been repeatedly documented during the last decade in different areas of the Mediterranean Sea. Moreover, this species occurs all over the world: along the Spanish coast (Delgado et al., 1990; Giacobbe et al., 1996; Vila et al., 2001), French coast (Nezan and Piclet, 1991), Adriatic Sea (Honsell, 1993), French Brittany coasts (Atlantic and English Channel), Ireland, England, and Denmark (Nehring, 1998; Hansen et al., 2003), South Australia (Hallegraeff and Lucas, 1988), India (Godhe et al., 2000), Malaysia (Usup et al., 2002), and North Sea (Nehring, 1998; Elbrächter, 1999; Hansen et al., 2003). *A. minutum* is heterothallic; that is, two different strains with compatible mating types (±) are required to form sexual resting cysts in this species. The obligatory dormancy period before germination can proceed is 1–1.5 months (Figueroa et al., 2007). Planozygotes are morphologically very similar to the vegetative stage, but in *A. minutum* their formation can be specifically measured in culture using flow cytometry (Figueroa et al., 2007).

Parvilucifera is a genus of dinoflagellate parasites that combines the features of dinoflagellates and apicomplexans, forming a 'missing link' between these two groups (Norén et al., 1999). *P. sinerae* has been recently described as a new species (Figueroa et al., 2008), different from *P. infectans* (Norén et al., 1999) and *P. prorocentri* (Leander and Hoppenrath, 2008). Cellular infection with *P. sinerae* is marked by the appearance of a flagellated zooid, which penetrates the cell and then multiplies within it. This leads to the death of the cell and its transformation into a spherical dark body called a sporangium. After a brief maturation time (around 48 h) the sporangium ruptures, thereby liberating as many as 400 swimming zooids (Figueroa et al., 2008) (Fig. 1).

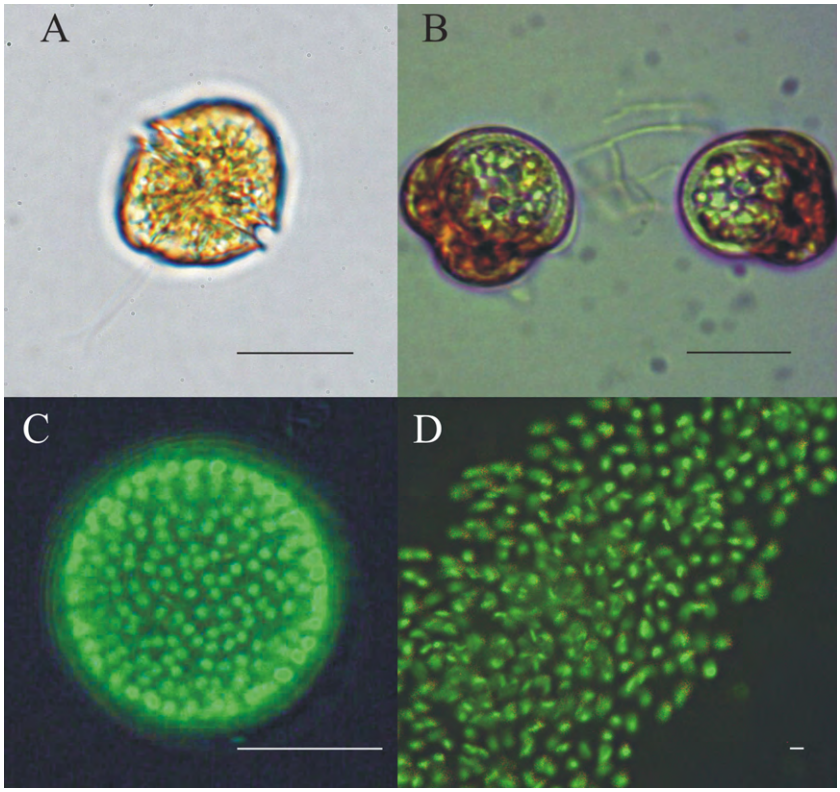

Fig. 1. Process of infection of *A. minutum* by *P. sinerae*. (A) Vegetative stage of *A. minutum*; (B) immature stage of infection; (C) mature sporangium after Sybr green staining, showing the zooids' nuclei; (D) released parasite swarmers after nuclear staining with Sybr green. Scale bars 10 μm in (A)–(C); 2 μm in (D).

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In the present study of the reproductive behavior of *A. minutum* crosses infected by *P. sinerae*, we found that *A. minutum* showed different susceptibilities to the infection depending on the geographical origin of the strain. Also, we verified that the host sexual cycle was modified when the parasite was present, because resting cyst formation, thought to be the main defense of the host against the parasite, was mostly avoided. This response was, however, dependent on the parental strains' level of resistance against the parasite and on the degree of their sexual compatibility.

2. Materials and methods

2.1. Culture maintenance and growth

Experiments were conducted with seven clonal strains of *A. minutum* obtained from the culture collection of the Centro Oceanográfico, Vigo, Spain (Table 1). A natural sample from the same locality of the parasite isolation was also tested for infectivity. Cultures were grown at 24 °C with a photoperiod of 12:12 h (light:dark) cycle. Illumination was provided by fluorescent tubes with a photon irradiance of 90 μmol photons m^{-2} s^{-1}. Culture stocks were maintained in Erlenmeyer flasks filled with 50 mL of L1 medium (Guillard and Hargraves, 1993) without silica. The medium was prepared with Atlantic seawater and adjusted to a salinity of 31 psu by the addition of sterile bi-distilled water.

The *P. sinerae* parasite culture was established after the isolation of sporangia that was formed in a natural *A. minutum* bloom on the Catalan coast of Spain (NW Mediterranean Sea). The culture was maintained in sterile polystyrene Petri dishes (Iwaki, Japan, 16-mm diameter) containing 10 mL of exponentially growing cells of *A. minutum* strain A10. Every 6–7 days, 20–25 sporangia were inoculated into fresh A10 cultures.

2.2. Sexual crosses and infection experiments

Self- and out-crosses were conducted in 1 mL of medium with no added phosphates (L-P) in duplicate sterile polystyrene Petri dishes (Iwaki, Tokio, Japan, 10.2-mm diameter) inoculated with exponentially growing cells (3000–4000 cells mL^{-1}) to a final concentration of 300 cells mL^{-1} (150 cells mL^{-1} of each parental strain). Although self-crosses were not expected to result in cyst production (Figueroa et al., 2007), they were nonetheless carried out to study vegetative growth under the same conditions used in the out-crosses for sexual induction (i.e., absence of phosphates).

To infect the cultures, newly formed parasite sporangia were individually isolated and washed in several drops of seawater. The dinoflagellate strains were classified as susceptible (S), low susceptible (LS), or resistant (R) to infection based on the infectivity of a parasite inoculum of 10 sporangia mL^{-1}. The number of sporangia that formed in each plate of an infected culture was counted at least once a day for 10 days. Putative R or LS cultures (self- and out-crosses) were infected twice in replicate ($n = 4$) to confirm resistance. Only mature sporangia (dark, round, and benthic) were counted because early stages of infection are difficult to detect and therefore cannot be accurately quantified. The abundance of mature sporangia was used as a proxy for the number of infected cells (Figueroa et al., 2008). Doubling times for sporangia duplication were calculated after 4 days, when the number of second-generation sporangia has been shown to be negligible (Figueroa et al., 2008). Cultures were examined under a Leica-Leitz DM-IRB inverted microscope (Leica Microsystems GmbH, Wetzlar, Germany) and micrographs were taken with a ProgRes C10 Plus digital camera (JENOPTIK Laser, Optik, Systeme GmbH, Germany). Sybr green staining was performed according to Figueroa et al. (2008).

2.3. Parasite growth rate

The parasite growth rate was estimated taking into account the increment of mature sporangia over time. As described by Guillard (1973), species-specific net duplication rates (day^{-1}) from each duplicate in susceptible strains and from each quadruple in resistant strains were calculated from the slopes of the regression lines of ln (N) vs. time, with N as the mean sporangium abundance during the time period of maximum sporangia production; $K = \ln(N_1/N_0)/(t_1 - t_0)$, and the duplication time for the sporangia population is $Kd = \ln 2/k$.

2.4. Heritability of resistance

To test whether the resistant genotype had been inherited by the offspring, 10 planozygotes resulting from a non-infected cross 651 × 663 (R × S) were identified by the presence of two longitudinal flagella and individually isolated in sterile polystyrene Petri dishes (Iwaki, 6.4-mm diameter) filled with L1 medium; after the planozygotes had divided, 21 clonal strains were isolated. Twenty-five days later, these cultures were infected and then checked for sporangia formation as described above.

2.5. Zygote formation

The number of planozygotes formed in the sexual out- and self-crosses was estimated according to the method of Figueroa et al. (2007). The method takes advantage of the light-induced synchronization of cell division in *Alexandrium* in order to calculate zygote formation by flow cytometry. Since *A. minutum* cells arrest in the G1 phase of the cell cycle after 48 h of darkness, diploids ($2n$) formed in a synchronized sexual cross-sampled immediately after the end of the dark period are zygotes and not dividing cells containing double the amount of DNA. *A. minutum* strains 651 × 653 (Table 1) were sexually crossed in duplicate 50-mL flasks (Iwaki, Tokio, Japan) under the same conditions as described above and then infected by individual isolation of sporangia 2 days after inoculation until a final concentration of 10 sporangia mL^{-1}.

Table 1
Isolation and history of the dinoflagellate *Alexandrium minutum* strains. Strains from the Atlantic Sea are marked in grey whereas Mediterranean strains do not.

Strain name	Key	Source	Year of isolation	Clonal	Self-compatibility (resting cyst production)
VGO 657	657	Port Saint Hubert (Brittany, France)-Atlantic Sea	2003	Yes	No
VGO 653	653	"	2003	Yes	No
VGO 650	650	"	2003	Yes	No
VGO 651	651	"	2003	Yes	No
VGO 652	652	"	2003	Yes	No
VGO 663	663	Ría de Vigo (Spain)-Atlantic Sea	2003	Yes	No
Palmira 2	P2	Mallorca (Spain)-Mediterranean Sea	2003	Yes	No
Arenys	AR	Arenys de Mar (Spain)-Mediterranean Sea	2006	No	Yes

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These cultures were synchronized as follows: after completion of the 48-h 12L:12D cycle, the incubators lights were turned off for 48 h. Samples for DNA and cell concentration determinations were taken from duplicates once the illumination cycle had been restored ($t_{0\ h}$ = 0 h of light after synchronization or $t_{8\ h}$ = 8 h of light after synchronization). Duplicate cultures were sampled at 4 and 6 days after inoculation.

For flow cytometric analyses, a 20-mL culture aliquot was fixed with 1% paraformaldehyde for 10 min and washed in PBS pH 7 (Sigma–Aldrich, St. Louis, USA) (1200 × *g* for 10 min). The pellet was resuspended in 5 mL of cold methanol and stored for 12 h at 4 °C to extract chlorophyll. The cells were then washed twice in PBS (pH 7) and the pellet resuspended in a staining solution (PBS, 3 μg propidium iodide mL^{-1} and 1.1 μg RNaseA mL^{-1}) for at least 2 h before analysis. The samples were analyzed in a Becton and Dickinson FACScalibur bench machine with a laser emitting at 488 nm. The flow rate was approx. 18 $\mu L\ min^{-1}$ and data were acquired in linear and log modes until around 10,000 events had been recorded. As an internal standard, 10 μl of a $10^6\ mL^{-1}$ solution of yellow-green 0.92 μm Polysciences latex beads was added to each sample. Fluorescence emission of propidium iodide was detected at 617 nm. ModFit LT (Verity software House) was used to compute peak numbers, coefficients of variation (CVs), and peak ratios for the populations' DNA fluorescence distributions. The settings for the DNA analyses ($n/2n$) of the *A. minutum* cells were previously established in non-infected cultures (Figueroa et al., 2007), assuring that the detection of sporangia by flow cytometry did not result in fluorescence overlap.

2.6. Statistical analyses

Statistical analyses were performed using STATISTICA 8.0 for Windows. The susceptibility of a sexual cross was analyzed using one-way ANOVA.

3. Results

The results can be summarized as follows:

(1) Clonal strains of *A. minutum* (Table 1) differed in their susceptibilities to parasitic infection (Table 2 and Fig. 2). The strains were classified as susceptible (S), low susceptible (LS), or resistant (R) depending on the doubling times (dt) of the population of the parasite sporangia (Table 2). Both the clonal Mediterranean strain studied and the natural sample from the Mediterranean were susceptible (S, dt > 0.4), and the populations were extinct 5 days post-infection. In contrast, Atlantic strains exhibited a low level of susceptibility (LS, dt > 0.05 < 0.4) or were resistant (R, dt < 0.05). LS cultures were able to sustain a low level of infection as well as sporangia formation without becoming extinct during a 7–9-day period. Resistant cultures showed no signs of infection and the sporangia used to infect them gradually became empty.

(2) R × S crosses gave rise to cultures infected at low levels (Table 2). This result was further analyzed by testing the resistance of the planozygotes' offspring. In all cases, strains (n = 21) established from the division of R × S planozygotes (F1 offspring) expressed a resistant or low susceptible genotype.

(3) The percentages of planozygotes formed 4 and 6 days after sexual crossing (approximately the time range in which the maximum for planozygote formation is expected, according to Figueroa et al., 2007) were comparable in infected and non-infected R × S cultures (ANOVA, $p < 0.05$); with a maximum percentage of planozygotes of 8.5–10% at day 4.

(4) However, resting cyst production was significantly lower in infected than in non-infected (control) R × S cultures (ANOVA, $p < 0.01$, Table 3), given that almost no planozygotes underwent resting cyst formation in the infected cultures.

(5) Compatible S × S crosses were infected significantly faster than was the case for each of the parental strains growing asexually (ANOVA, $p < 0.01$)(Table 2 and Figs. 3 and 4a).

(6) The degree of sexual compatibility among the parental strains determined both the infection rate and survival. Cell survival 12 days post-infection was significantly higher (ANOVA, $p < 0.01$) in R × S crosses with a high level of compatibility than in those crosses in which compatibility was low (Fig. 4b).

(7) Pellicle (asexual) cysts, as described by Bravo et al. (2010), were not long term resistant to parasite infection, since their presence was not observed in the infected cultures after all mobile stages had been killed (5–7 days after infection).

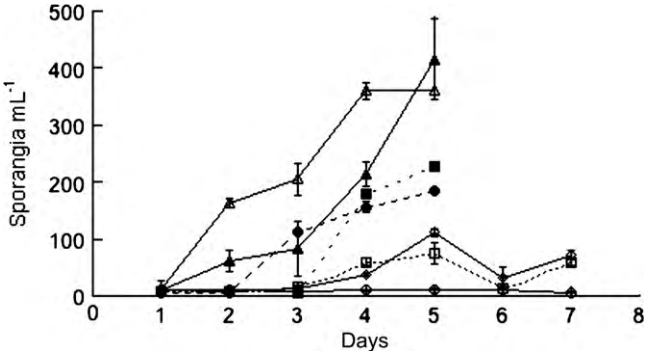

Fig. 2. Graph representing the different rates of sporangia formation (average and standard deviation) in different *A. minutum clonal* cultures. Each culture was crossed with itself (self-cross) to later allow comparison of the data with those resulting from out-crosses (induction of sexuality): (▲) p2xp2, (△) 663 × 663, (■) 650 × 650, (●) 657 × 657, (○) 651 × 651, (□) 652 × 652, (◇) 653 × 6553. See Table 1 for information on the origin of each parental strain.

Table 2
Doubling times of *P. sinerae* sporangia (average ± standard deviation) in different dinoflagellate cultures (n = 4).

Culture	Origin	Susceptibility	Doubling time (dt) average ± SD
AR	M	S	0.70 ± 0.26
P2	M	S	0.52 ± 0.02
663	A	S	0.92 ± 0.08
650	A	S	0.56 ± 0.02
657	A	S	0.49 ± 0.07
653	A	LS	0.12 ± 0.02
652	A	LS	0.15 ± 0.04
651	A	R	0
650 × P2	A × M	S × S	1.02 ± 0.02
663 × P2	M × M	S × S	0.57 ± 0.18
651 × P2	A × M	R × S	0.02 ± 0.06
651 × 663	A × M	R × S	0.02 ± 0.03

S = susceptible (dt > 0.4); LS = low susceptible (dt > 0.05 < 0.4); R = resistant (dt < 0.05); A = Atlantic; M = Mediterranean.

Table 3
Production of *Alexandrium minutum* resting cysts (average ± standard deviation) in infected and non-infected compatible sexual crosses 25 days after being inoculated (n = 4). See Table 1 for the complete information on the parental strains.

Sexual crosses between clonal parental strains (susceptibility)	Resting cyst production Non-infected culture (cysts mL^{-1})	Resting cyst production Infected culture (cysts mL^{-1})
650 × 650 (R)	134 ± 21.2	2.0 ± 1.4*
651 × 653 (R)	62.0 ± 8.5	7.0 ± 2.8*
P2 × 650 (S)	2.3 ± 1.4	0

* Significantly different between non-infected and infected cultures (ANOVA, $p < 0.01$).

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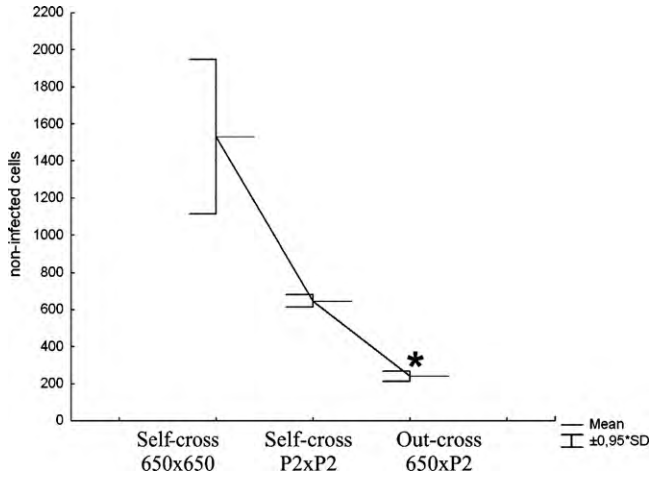

Fig. 3. The increased susceptibility to parasite infection of a cross between susceptible and sexually compatible parents compared with each self-cross (asexual). *The number of non-infected cells in the out-crosses is significantly different from that in the self-crosses (ANOVA, $p < 0.01$).

4. Discussion

The results of the present study suggest that sexual crosses of *A. minutum* exposed to the Perkinsozoid parasite *P. sinerae* shift their life-history strategy towards more rapid production of recombinant offspring by increasing the division rates of mobile zygotes (planozygotes) and avoiding long-term encystment. This scenario requires that the planozygote has a genotype resistant to parasite infection and is thus able to carry out meiosis. If this is not the case, then asexual reproduction (mitotic) is more advantageous, because susceptible planozygotes may not be able to either divide or form resting cysts. Strongly supporting this theory is our observation that the number of resting cysts formed in resistant cultures was surprisingly close to zero, even when these cultures gave rise to hundreds of sexual resting cysts in the absence of the parasite. Moreover, *A. minutum* resting cysts are completely resistant to parasite infection, regardless of the susceptibility of the parental strains.

Our initial experiments confirmed local adaptation of the parasite since all Mediterranean strains were easily infected, as also observed by Figueroa et al. (2008) and Llavería et al. (2010), whereas Atlantic strains were slowly infected or totally resistant. The ability of parasites to infect hosts from local populations more efficiently than those from distant populations is consistent with the negative frequency-dependent selection predicted by the matching-allele model, in which co-evolution of the host–parasite system is presumed to be dependent on sex and recombination (Howard and Lively, 1994; Lively, 1989; Lively and Dybdahl, 2000).

In this study, strains of *A. minutum* were classified in three categories (susceptible, low susceptible, and resistant) according to the time needed for the mature sporangia to double their population 4 days after infection of the respective culture. Whereas resistant cultures of *A. minutum* VGO 651 were not infected at all, i.e., no new sporangia appeared in the culture, it is uncertain why other cultures showed very low infection levels. There are two possible explanations: either the infection of these cultures by the parasite was, for some reason, more difficult, or the growth rate of the parasite in these cells was slower. Whatever the reason, the difference in the susceptibilities of the host strains was real and has

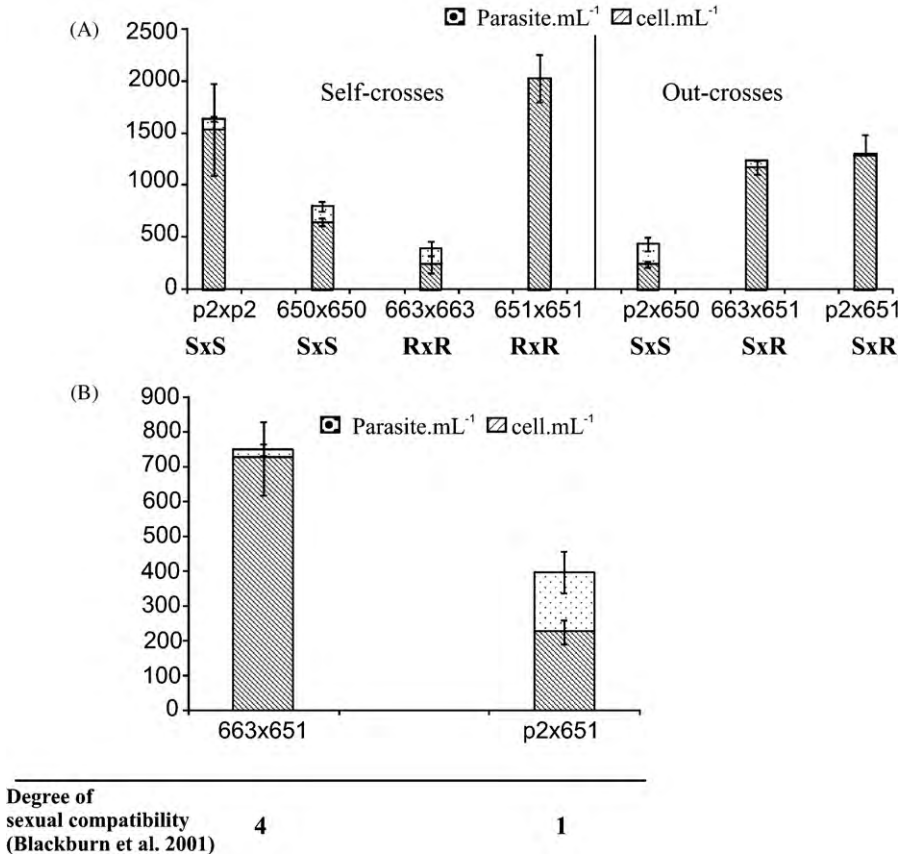

Fig. 4. Number of parasite sporangia (parasite mL^{-1}) and cell survival (cells mL^{-1}) at 5 (a) and 12 (b) days after parasite infection. The degree of sexual compatibility reflects the production of cysts of non-infected cultures, based on Blackburn et al., 2001: 4 = $>1.0 \times 10^5$ cysts L^{-1}; 1 = 3.0×10^2–2.0×10^3 cysts L^{-1}.

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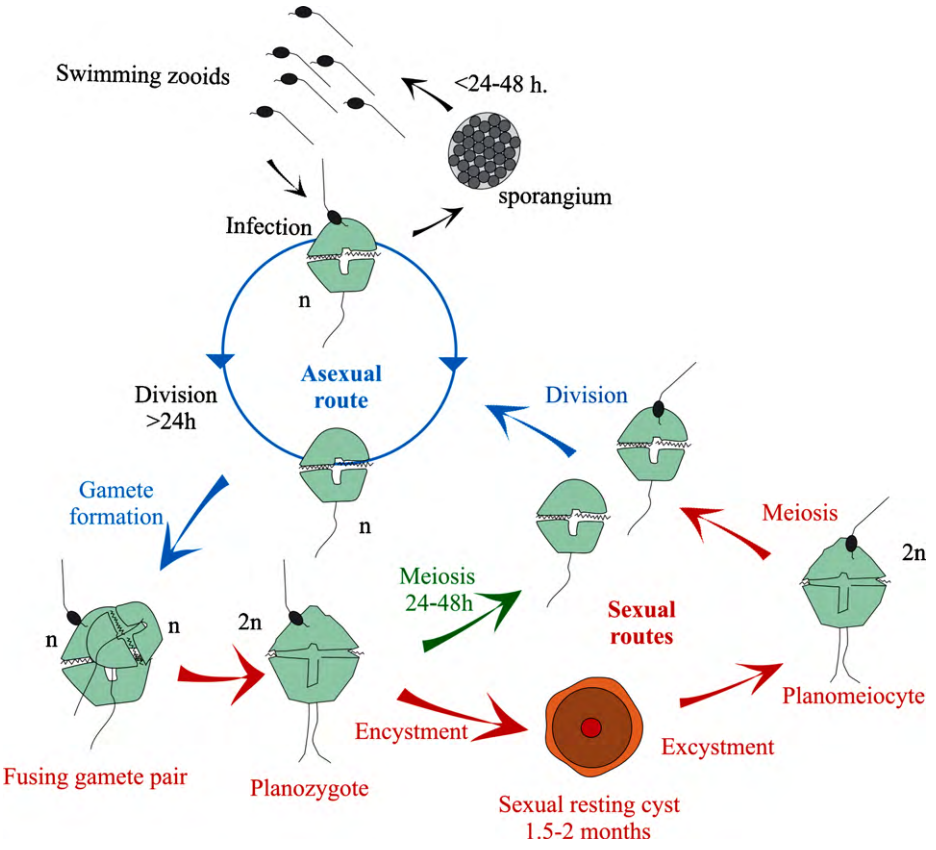

Fig. 5. *Alexandrium minutum* and *Parvilucifera sinerae* life cycles (based on Figueroa et al., 2007 and Figueroa et al., 2008 respectively).

important ecological consequences. Furthermore, susceptibility not only varied with the clonal parental strains, but also depended on the presence or absence of sexual processes in the cultures, an observation that we now discuss.

In dinoflagellates, sex is facultative (they can also grow exclusively by mitosis) and is initiated by the fusion of compatible gametes to form a mobile zygote (planozygote). In many studied species, such as *A. minutum*, the planozygote can follow one of two different sexual routes: either division or the formation of a stress-resistant dormant form (resting cyst) (Fig. 5). The planozygote can therefore be used as a checkpoint for testing whether a particular factor promotes or inhibits a change in the sexual recombination rate; that is, whether the planozygote divides (Fig. 5, green) or produces resistant cysts (Fig. 5, red) in response to an external environmental challenge (Figueroa et al., 2006a,b, 2007).

The results of this study showed that resting cyst production was significantly lower in infected than in non-infected (control) R × S cultures. Together with the fact that planozygotes were detected in the infected cultures at days 4 and 6 after sexual crossing (the time at which maximum zygote production in *A. minutum* is reached, according to Figueroa et al., 2007), the lack of cysts in infected cultures suggests the occurrence of high rates of planozygote division (Fig. 5, green).

This conclusion is supported by two additional observations: resting cysts were not formed in the infected cultures, and cultures established from the division of planozygotes isolated from R × S cultures were likewise resistant. Why, if resting cysts are inherently resistant to the parasite (Toth et al., 2004, Figueroa et al., 2008), were they completely absent in R × S cultures? Two hypotheses can be proposed to answer this question: (1) a chemical cue may have prevented gamete fusion and planozygote formation and (2) the planozygotes divided. The first hypothesis is ruled out by the observation of high numbers of fusing gamete pairs and planozygotes in the cultures, as shown in Figueroa et al. (2008). Furthermore, flow cytometry analyses detected the presence of zygotes in percentages very similar to those recorded in the non-infected cultures, at least in the two measurements that were made (additional measurements were not possible due to the low number of swimming *A. minutum* cells remaining once the infected cells reached the exponential phase). Thus, sexuality was not inhibited in the infected cultures. The second hypothesis, that the planozygotes divided, is supported by our results on individual, isolated planozygotes and the relation between resistance and sexual compatibility among the parental clonal strains. Both aspects are expanded upon below.

Although sex in dinoflagellates may take place within a clonal strain (homothallism), the strains used in the present experiments had to be crossed with other compatible clonal strains to obtain sexuality (heterothallism). However, some *A. minutum* crosses produce many more planozygotes and resting cysts than others (Figueroa et al., 2007). Cell survival 12 days post-infection was significantly higher in the R × S cross with a high level of compatibility than in the cross in which compatibility was poor. This graded sexual compatibility allowed us to infer that the rate of sexual recombination increased in all compatible crosses, although the benefits were greater for those strains with higher degrees of sexual compatibility. Both the (R × S) planozygotes and the offspring resulting from their division were almost completely resistant to the parasite, although low levels of sporangia formation were detected in some of the cultures. Given that dinoflagellates are haploid, this finding could be explained by assuming that resistance is a quantitative trait controlled by several genes or is due to maternal effects (Lynch and Ennis, 1983).

The immediate consequence of planozygote division (Fig. 5, green) and therefore bypassing of the encystment route (Fig. 5, red) is a higher rate of sexual recombination. Meiosis in dividing

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planozygotes was recently demonstrated (Figueroa et al., 2006b, 2007), and recombinant offspring are produced much faster by zygote division than by the formation of a resting cyst, which must undergo a period of dormancy before germination and meiosis can occur. Thus, planozygote division may represent an energy-saving strategy because a resting cyst is no longer needed once a resistant zygote has been formed. Division is also chosen over encystment when planozygotes are placed in nutrient-enriched medium (sexuality is induced in laboratory conditions by removing nutrients from the culture medium). The presence of nutrients may be a signal indicating the end of stressful conditions and, accordingly, that resting cyst formation is no longer needed (e.g., Figueroa and Bravo, 2005b; Figueroa et al., 2006a). Similarly, if one of the strains involved in the sexual cross has a resistant genotype ($S \times R$ or $R \times R$), the planozygote is parasite-resistant, and sexual recombination takes place faster than in non-infected cultures because zygote division is preferred over cyst formation, the fate of most planozygotes in non-infected cultures.

Another interesting result of this study is that $S \times S$ crosses became infected faster than each parental strain growing separately exclusively by mitosis (asexually). The hypothesis is that no sexual route for the susceptible strains is convenient to avoid infection in a short-term. Sexuality implies gamete fusion and planozygote formation, and, consequently, temporary changes in thecal and cell membrane properties that may also facilitate a more rapid infection. This case may be similar to that described for viral infection of the coccolithophore *Emiliania huxleyi*, in that a drastic difference in viral susceptibility between life-cycle stages with different ploidy levels was reported (Frada et al., 2008). Additionally, although the resistance of resting and pellicle cysts to infection is considered to be the main defense mechanism employed by dinoflagellates against parasitic attack (Delgado, 1999; Toth et al., 2004), this strategy is useless for susceptible $S \times S$ crosses at parasite densities such as those employed in this study. Firstly, cultures of planozygotes became totally infected before planozygote encystment was completed. Secondly, pellicle cysts were not observed after 5–7 days, which implies that even if they had formed (pellicle cysts were not counted in the first days of infection) they were quickly infected. However, this higher susceptibility of the $S \times S$ crosses offers a plausible explanation as to why gamete mating is not triggered by the presence of the parasite. Nonetheless, Mediterranean strains can still benefit from sexuality if the parasite density allows for a certain amount of successful resting cyst formation. Here, it must be pointed out that a given parasite density will be much less efficient infecting *A. minutum* in a natural bloom than in culture conditions, due to the existence in the former of parasite predators (zooplankton) and turbulent conditions that delay the parasite–host initial infection (Llavería et al., 2010).

Our results showed that sexual crosses between susceptible and resistant strains lead to an increase in resistance that is proportional to the sexual compatibility (i.e., the recombination rate) of the parental strains, but they do not rule out the advantages of asexual growth during a parasitic attack. In fact, the suitability of sexuality depends on the partner genotype, which suggests that the advantage conferred by sex makes it the preferred response to parasitic attack only in the presence of the appropriate partner (Pomiankowski and Bridle, 2004).

Sex occurs in almost all eukaryote groups. However, asexual species occur, showing that asexuality has evolved many times, and that there must be some factor maintaining it, because sexuality has the disadvantage that it is costly (see for review Agrawal, 2006). Similarly, if the costs of cellular defense strategies are considered, then the mode of resistance selected may be related to the resources that are currently available (Moret and Schmid-Hempel, 2000; Rigby and Jokela, 2000). Accordingly, the proportion of sexual forms in a population would depend on the balance between the advantage gained through fluctuating epistasis and the energy costs of sexual reproduction (Lythgoe, 2000). Dinoflagellates have no costs related to male-female differentiation or parental care, and only in some cases are costs incurred due to mating preferences. Nonetheless, these organisms show phenotypic plasticity related to sexual reproduction not by increasing mating frequency, but by increasing the percentage of planozygotes undergoing division, i.e., the sexual recombination rate. This result supports the hypothesis saying that to resist parasites, hosts must continually change gene combinations and preserve not one ideal genotype but rather an array (Hamilton et al., 1990).

The present results have immediate practical consequences. First, while strain-specific host resistance has never been documented in toxic strains of algae, foreign strains could nonetheless readily invade new waters because, analogous to frequency-dependent selection, a resident parasite should be most harmful to its endemic host. Thus, if Atlantic strains of *A. minutum* were introduced into an area of the Mediterranean Sea, they would outcompete Mediterranean strains, assuming the lack of a natural competitor, and cause a presumably larger bloom. Second, parasites of the genus *Parvilucifera* have been suggested as a resource to control harmful algal blooms (Delgado, 1999; Moestrup and Norén, 2003). As shown by our results, to be effective, this type of biocidal strategy must consider the genetic structure of the blooming population and be based on an in-depth understanding of its sexual processes in natural environments. Currently, adequate knowledge regarding either of these aspects is still lacking.

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